Distribution of Major Polyphenolic Compounds in Vine Grapes of Different Cultivars Growing in South Moravian Vineyards

Ondřej MIKEŠ[1], Naděžda VRCHOTOVÁ[2], Jan TŘÍSKA[2], Marie KYSELÁKOVÁ[1] and Jan ŠMIDRKAL[3]

[1]Department of Viticulture and Enology, Faculty of Horticulture, Mendel University of Agriculture and Forestry in Brno, Lednice, Czech Republic; [2]Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic; [3]Department of Dairy and Fat Technology, Faculty of Food and Biochemical Technology, Institute of Chemical Technology in Prague, Prague, Czech Republic

Abstract

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The main chemoprotective polyphenolic compounds in the *Vitis vinifera* berries, rachis, and pedicels of 10 cultivars classified for the production of wine and growing in Southern Moravian vineyards, the Czech Republic, were studied. The following compounds were determined in the frozen fresh berries: gallic acid (1.8–13.3 mg/kg), catechin (70.3–659.1 mg/kg), epicatechin (67.1–237.2 mg/kg), *trans*-resveratrol (0.1–1.5 mg/kg), and pterostilbene (in traces); in the freeze-dried rachis and pedicels: rutin (10.5–68.6 mg/kg), isoquercitrine (29.8–218.3 mg/kg), catechin (283.7–2227 mg/kg), epicatechin (47.2–215.2 mg/kg), *trans*-resveratrol (2.6–37.1 mg/kg), and pterostilbene (0.01–0.13 mg/kg), respectively. The contents of polyphenolic compounds were different in various cultivars. The highest levels of catechin and epicatechin were found in the grapes of cv. Blauer Burgunder (3195 mg/kg), in which the second highest content of *trans*-resveratrol (33.2 mg/kg) was also found. The content of pterostilbene in the whole berries or stems was estimated for the first time. The rachis and pedicels could serve as a prospective source of polyphenolic compounds.

Keywords: *Vitis vinifera* L.; cultivars; polyphenolic compounds; berries; stems; HPLC

While polyphenolic compounds in wine and their positive effects on human health have been the subject of many scientific studies and papers for more than ten years making the public familiar with the topic, papers dealing with the determination of these compounds in vine grapes are scarce.

Polyphenolic compounds are secondary metabolites naturally present in vine grapes which are

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released during the vinification process. They participate in the plant metabolism and are responsible for the plant growth regulation. As phytoalexins, they protect plants against infections and the attack by microorganisms (Ecarpa & Gonzalez 2001; Matějíček *et al*. 2003). The contents of polyphenolic compounds in vine grapes depend on the variety, geographical location (climate, intensity of solar radiation, etc.), viniculture technologies or stress factors occurring during growing and ripening of the grapes. The contents of polyphenolic compounds in wine depend especially on the mode of vinification. Their concentrations in red wines are up to 20 times higher in comparison to their contents in white wines (Siemann & Creasy 1992; Jeandet *et al*. 1995; Goldberg *et al*. 1999; Faustino *et al*. 2003).

Chemoprotective polyphenolic compounds are represented by stilbenes, flavonoids, and phenolic acids, the structure of the selected compounds is presented in Figure 1.

Resveratrol is present mostly in the grape skin. Its content varies in different varieties of *Vitis vinifera* as well as in different cultivars (Soleas *et al*. 1997). Resveratrol is found in different concentrations in various parts of *Vitis vinifera*. The stems of *Vitis*

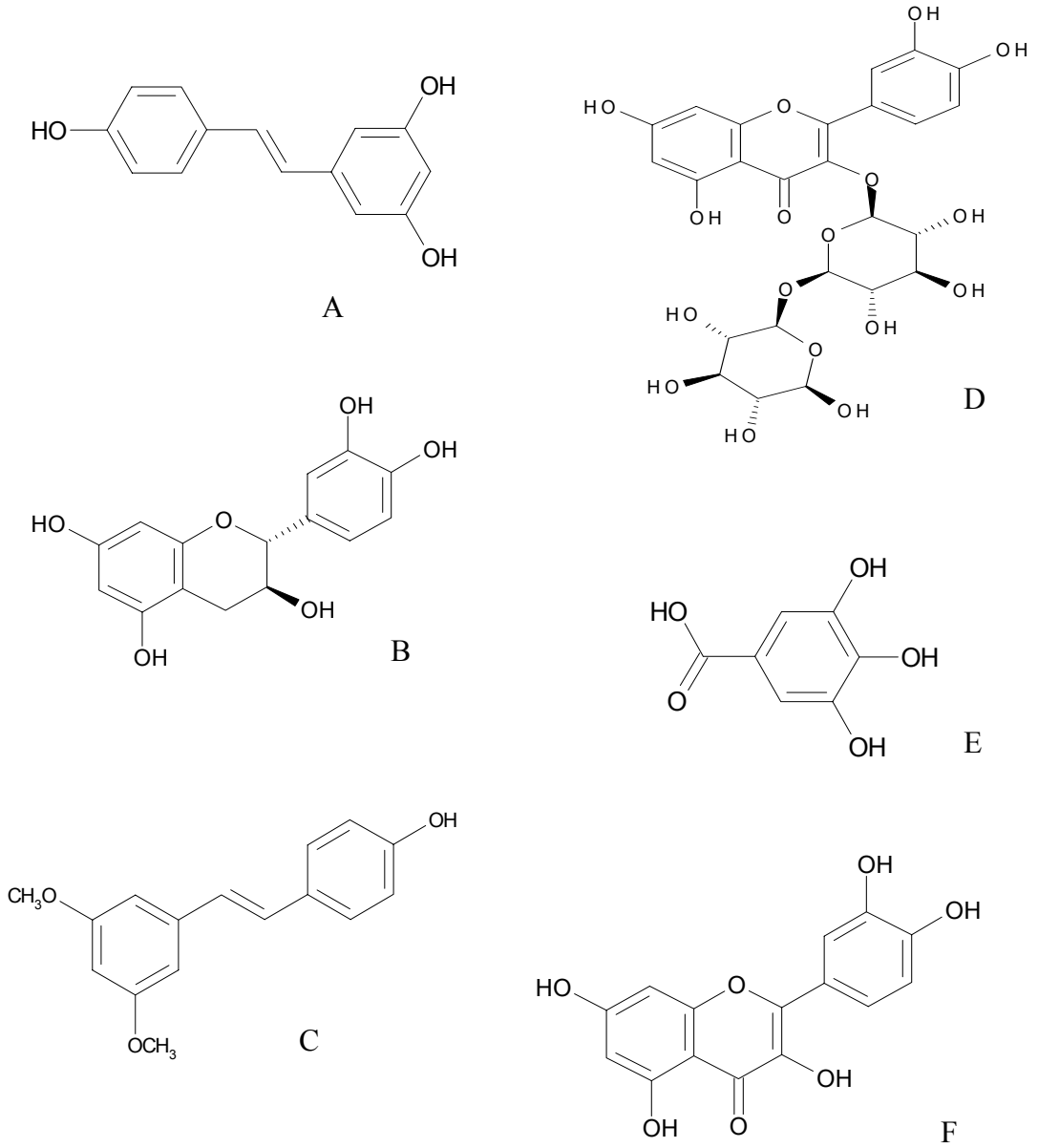

Figure 1. The structure of the selected polyphenolic compounds: *trans*-resveratrol (A), catechin (B), pterostilbene (C), rutin (D), gallic acid (E), quercetin (F)

vinifera were found to be the richest source of resveratrol, its content reached up to 500 mg/kg dry matter (Melzoch *et al*. 2001). The concentration of *trans*-resveratrol in wines ranges from 0.1–10 mg/l. During the ripening process of grapes, the amount of resveratrol increases progressively. In the plant materials, a mixture of *trans*- and *cis*-forms is naturally present, while *trans*-isomer usually predominates. Both forms are also present as glycosides (piceids) in the plant materials. Both *trans*- and *cis*-piceids have been studied in the grape skins and stem tissues of three *Vitis vinifera* varieties (Sun *et al*. 2006). *Cis*-resveratrol is not present in the vine grapes (especially in the grape skins) but forms in the course of the initial phase of alcoholic fermentation as a product of *trans*-resveratrol isomerisation, or as a product of its polymers decomposition (Roggero & Garcia Parrilla 1995; Soleas *et al*. 1997). According to the recent literature (Otreba *et al*. 2006), *cis*-resveratrol may arise as a natural means of defence against fungal infection under organic farming conditions. Resveratrol (as an antioxidant) possesses anti-atherotrombic and vasodilatation functions (Bertelli *et al*. 1996) and inhibits the oxidation of low-density lipoproteins (LDL) (Belguendaz *et al*. 1998). The study published by Jang *et al*. (1997) has shown that resveratrol may prevent cancer by its antioxidant and antimutagenic activities.

The structure and properties of pterostilbene are similar to those of resveratrol. Unlike resveratrol, pterostilbene possesses anti-diabetic properties in addition. The content of pterostilbene in the grapes is about ten times lower compared to that of resveratrol (Rimando *et al*. 2002).

Quercetin is present in higher concentrations in vine grapes and is usually bound to a saccharide forming more stable glycosides (isoquercitrine, rutin etc.) (Dadáková *et al*. 2003). A relatively high content of quercetin was found in the grape pomace (Careri *et al*. 2003). The antioxidant properties of quercetin are complementary to those of resveratrol (Constant 1997) but it shows a prooxidant activity in some cases (Kessler *et al*. 2003). Quercetin possesses anti-atherotrombic effects; it reduces LDL oxidation and platelet aggregation (Hayek *et al*. 1997).

Catechin and the related epicatechin possess strong antioxidant effects and both are present in vine grapes and wine at the highest levels of all flavonoids (Revilla *et al*. 1991; Dadáková *et al*. 2003).

Amino acids, vitamins, mineral substances, other flavonoids, and alcohol in wine, are an other chemoprotective components of vine grapes and wine (Soleas *et al*. 1997).

The extraction of freeze-dried stems with methanol aimed at determining the relationship between the yields of catechin, epicatechin, gallic acid, and resveratrol, was studied earlier (Tříska *et al*. 2004) using different concentrations of methanol (60–90%, v/v). For the resveratrol extraction, 90% methanol was recommended, for that of catechin, epicatechin and gallic acid, 70–80% methanol was recommended in order to obtain the highest yields of the compounds.

It is very important to utilise the raw materials in every agricultural branch completely, not only from the environmental requirements point of view, but also for the economical reasons. Approximately more than 20% of the wine production process provide wastes, causing a serious environmental problem that has to be solved urgently. Recently, Arvanitoyannis *et al*. (2006) have proposed several procedures to solve this problem effectively. A very viable business has also emerged within the wine industry producing extracts from the grape seeds and grape skins as nutritional supplements (Shrikhande 2000). Yilmaz and Toledo (2006) have shown that health functional components of the grape skin and seeds are comparable to those of fruits and vegetables as concerns their antioxidant capacities.

Therefore, the main goal of the present work was the determination and comparison of the significant chemoprotective polyphenolic compounds contents in different parts (berries, rachis and pedicels) of the 10 most common cultivars officially classified as vine cultivars of *Vitis vinifera* for the production of wine and generally most widespread in the vineyards in Southern Moravia. The following chemoprotective polyphenolic compounds were selected for the study: *trans*-resveratrol, pterostilbene, catechin, epicatechin, gallic acid, rutin, and isoquercitrine, using for the analyses reversed-phase high performance liquid chromatography (HPLC) with diode array detection (DAD).

MATERIALS AND METHODS

Samples. Seven samples of healthy white grape cultivars (Grüner Veltliner, Malverina, Hibernal, Müller Thurgau, Rheinriesling, Welschriesling, and Neuburger) and three samples of healthy blue

grape cultivars (Blauer Burgunder, Lemberger and St.Laurent) were picked in the Gene-bank vineyard – *in situ* – in Lednice (Southern Moravia, the Czech Republic) at harvesting maturity (vintage 2004). Each sample consisted of three vine grapes from three different plants of the same cultivar. The berries were carefully separated from the stems without any undesirable injury to their skin surface. The berries and stems were stored in the dark in a freezer (–20°C) for two months until the next treatment. The final samples consisted of about 100 g of the berries and 20 g of the stems each.

Sample preparation. The frozen stems (see explanation above) were freeze-dried and powdered. 0.25 g of the powder was extracted with 3 ml of 90% methanol (v/v) for 30 minutes. After centrifugation (1990 g, 10 min), the sediment was washed twice with 1 ml of 90% methanol. The combined supernatants were stored in a refrigerator (–20°C) until the injection into a HPLC system for the analysis for catechins, rutin, isoquercitrine, and resveratrol. The supernatants were left to thaw in the dark at room temperature and injected immediately after becoming liquid.

0.25 g of the powder was extracted with 2 ml of ethyl acetate for 30 minutes. The ethyl acetate fraction was filtered using cotton-wool. The sediment was washed twice with 1.5 ml of ethyl acetate and filtered. The combine filtrates were dried with a nitrogen gas stream and the compounds were finally diluted with 0.2 ml of 100% methanol. The final solution was stored in a refrigerator (–20°C) until the injection into the HPLC system for the determination of pterostilbene.

The frozen berries (25 g) were mixed with 60 ml of cold (–20°C) 80% methanol (v/v). After 30 min extraction, the mixture was centrifuged (1990 g, 10 min) and the sediment was washed twice with 10 ml of 80% methanol. The homogenised sample of the combined supernatants was stored in a refrigerator (–20°C) until the injection into a HPLC system for the analysis for catechins, rutin, isoquercitrine, and resveratrol.

The frozen berries (25 g) were mixed with 25 ml of ethyl acetate and extracted by means of stirring for 60 minutes. The ethyl acetate solution was separated and 25 ml of ethyl acetate were added to the sediment, carefully stirred for 30 min and by centrifuged (1990 g, 10 min). The ethyl acetate fractions were collected and concentrated in a vacuum evaporator and finally diluted with 1 ml of methanol. Before the injection into the HPLC system for the determination of pterostilbene, the extract was stored in the dark in a refrigerator.

Reagents and solvents. *Trans*-resveratrol and rutin standards were obtained from Sigma-Aldrich (Prague, Czech Republic). *Trans*-pterostilbene was synthesised by J. Šmidrkal (Institute of Chemical Technology in Prague, Czech Republic). Catechin, epicatechin, and gallic acid were purchased from Sigma-Aldrich (Prague) and isoquercitrine, ethyl acetate, and *o*-phosphoric acid from Fluka (Sigma-Aldrich, Prague, Czech Republic). Acetonitrile and methanol were purchased from Merck (Prague, Czech Republic).

Chromatographic methods. The separations were carried out using Luna Phenomenex C18(2) column (2 × 150 mm, 3 μm, Torrance, CA, USA) and Hewlett Packard HPLC system (Wilmington, DE, USA), model 1050, with diode-array detector (model 1040A). The injected volume was 5 μl. Mobile phase: acetonitrile-orthophosphoric acid-water (5:0.1:94.9) as solvent A, and acetonitrile- orthophosphoric acid-water (80:0.1:19.9) as solvent B were used, respectively.

The separation of gallic acid, catechins, rutin, isoquercitrine, and *trans*-resveratrol was carried out using the following gradient: from 0% to 45% of solvent B in 55 min and from 45% to 100% in 10 min, with the flow rate of 0.25 ml/min.

The determination of pterostilbene was performed using a linear gradient from 20% to 100% of solvent B in 25 min, with the flow rate of 0.25 ml/min.

The column temperature was kept at 25°C. Gallic acid and catechins were detected at 220 nm, rutin, isoquercitrine, *trans*-resveratrol, and pterostilbene at 315 nm.

RESULTS AND DISCUSSION

Generally, there is a limited number of papers dealing with the determination of chemoprotective compounds in vine grapes and, in addition, the literature has described very different samples, i.e. of different varieties, cultivars, parts of vine grapes, as well as different pre-treatments. Therefore, the majority of the published data including our results are difficult to compare. In our study, we have determined the following polyphenolic comounds: catechin, epicatechin, *trans*-resveratrol, pterostilbene, gallic acid, rutin, and isoquercitrine in both berries (Table 1) and stems (rachis and pedicels) (Table 2). The contents of pterostilbene, rutin, and isoquercitrine in berries (Table 1) were

Table 1. The concentrations of the selected polyphenolic compounds in berries of ten cultivars of *Vitis vinifera* (mg/kg f.w.)

Cultivar	Catechin	Epicatechin	*Trans*-resveratrol	Gallic acid
Grüner Veltliner	659.1	122.9	0.1	3.9
Hibernal	532.9	172.7	0.3	4.0
Malverina	242.0	100.0	0.3	3.5
Müller Thurgau	70.3	67.1	0.3	2.6
Rheinriesling	179.6	89.2	0.2	2.1
Welschriesling	103.3	80.8	0.5	1.8
Neuburger	90.1	114.8	1.5	3.9
Blauer Burgunder	515.6	237.2	0.5	5.2
Lemberger	197.5	204.8	0.3	6.7
St. Laurent	39.2	467.3	1.0	13.3

below the limit of detection (0.005 mg/kg f.w.) for pterostilbene. The contents of gallic acid in rachis and pedicels (Table 2) were also below the limit of detection (0.01 mg/kg f.w.). The precision of a set of three replicate results for all analytes expressed as R.S.D. is better than 3%.

The contents of catechin and epicatechin were the highest among those of all polyphenolic compounds studied in both parts of grapes. The maximum levels of catechin and epicatechin in the vine rachis and pedicels (Table 2) were found in Blauer Burgunder (2227 and 215.2 mg/kg f.w., respectively). In contrast, the cultivar Müller Thurgau contained the lowest concentrations of catechin and epicatechin in the berries (70.3 and 67.1 mg/kg f.w., respectively) out of all cultivars (Table 1), as well as in the rachis and pedicels (283.7 and 47.2 mg/kg f.w.) (Table 2). St. Laurent contained the highest amount of epicatechin in the berries (Table 1) of all the cultivars studied (467.3 mg/kg f.w.).

The contents of catechin in the interspecific hybrids found by Tříska *et al*. (2004) varied from 79.4 to 500 mg/kg f.w. in different whole berries, and from 100.1 to 1789 mg/kg f.w. in the stems. The contents of epicatechin ranged from 62.9 to 482.4 mg/kg f.w. in different grape be and from 10.4 to 315.7 mg/kg f.w. in the stems. The results of our study regarding epicatechin were similar but the levels of catechin found in the rachis and pedicels were higher (Table 2).

The difference between the chromatograms of the rachis and pedicels extract (A) and the berries extract (B) is shown in Figure 2.

Table 2. The concentrations of the selected polyphenolic compounds in stems of ten cultivars of *Vitis vinifera* (mg/kg f.w.)

Cultivar	Catechin	Epicatechin	*Trans*-resveratrol	Pterostilbene	Rutin	Isoquercitrine
Grüner Veltliner	1392.8	95.4	30.1	0.04	20.7	82.4
Hibernal	865.5	182.2	21.9	0.02	43.0	58.6
Malverina	440.3	60.1	12.4	0.01	40.1	218.3
Müller Thurgau	283.7	47.2	37.1	0.01	32.3	33.8
Rheinriesling	522.7	60.5	31.2	0.02	44.5	34.6
Welschriesling	1339.9	158.0	10.2	0.10	68.6	57.0
Neuburger	1116.7	115.8	2.6	0.01	32.8	29.8
Blauer Burgunder	2226.6	215.2	32.7	0.03	46.2	61.2
Lemberger	1682.6	177.1	4.2	0.13	10.5	103.6
St. Laurent	917.3	100.7	16.2	0.11	22.0	107.2

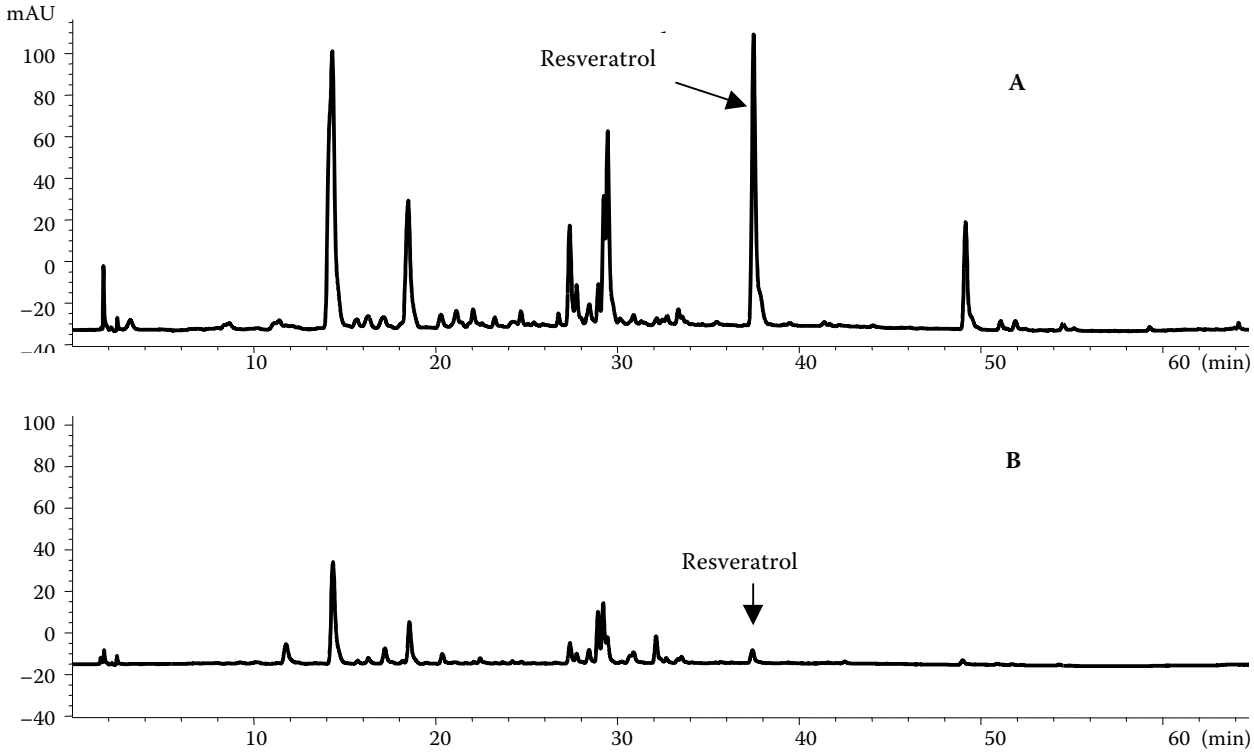

Figure 2. Chromatograms of vine rachis and pedicels (A) and berries (B) methanolic extracts (cultivar Welschriesling). Detection at 315 nm

The content of *trans*-resveratol in the berries (Table 1) was negligible. Neuburger berries revealed the highest concentration of *trans*-resveratrol (1.5 mg/kg f.w) of all cultivars. The rachis and pedicels (Table 2) of the same cultivar contained the lowest amounts of *trans*-resveratrol (2.6 mg/kg f.w). The highest levels of *trans*-resveratrol were found in the rachis and pedicels (Table 2) of cv. Müller Thurgau (37.1 mg/kg f.w.) and cv. Blauer Burgunder (32.7 mg/kg f.w.).

Different whole berries of the interspecific hybrids studied earlier by Tříska *et al.* (2004) contained from 0.2 to 9.4 mg/kg f.w. of *trans*-resveratrol, while the levels of *trans*-resveratrol in the stems ranged from 44.3 to 388.3 mg/kg f.w. Palomino *et al.* (2000) found 0.96 mg/kg f.w. of *trans*-resveratrol in whole berries. In comparison with the results of Tříska *et al.* (2004), we found lower contents of *trans*-resveratrol in the berries, rachis and pedicels, but still several times higher than those published by Palomino *et al.* (2000).

To the best of our knowledge, the contents of pterostilbene in whole berries and stems have never been determined before. We found that the content of pterostilbene in berries was at the detection limit level in all cultivars. Nearly one order of magnitude higher concentrations of pterostilbene were found in the rachis and pedicels (Table 2) of Lemberger (0.13 mg/kg f.w.), St. Laurent (0.11 mg/kg f.w.), and Welschriesling (0.10 mg/kg f.w.).

Gallic acid was not detected in the rachis and pedicels. The blue grapes showed higher contents of gallic acid than the white ones; blue grapes from 5.2 to 13.3 mg/kg f.w. and the white ones from 1.8 to 4.0 mg/kg f.w, respectively. (Table 1). The contents of gallic acid in different whole berries of interspecific hybrids found by Tříska *et al.* (2004) varied from 2.5 to 18.4 mg/kg f.w., while those in the stems varied from 2.4 to 13.8 mg/kg f.w. in comparison with the above mentioned results, we found slightly lower levels of gallic acid in the berries, but the contents of gallic acid in the rachis and pedicels were below the limit of detection.

The contents of isoquercitrine and rutin in the extracts from the berries were also below the limit of detection. The contents of rutin in the rachis and pedicels (Table 2) ranged between 10.5 mg/kg f.w (cv. Lemberger) and 68.6 mg/kg f.w (cv. Welschriesling). The highest levels of isoquercitrine in the rachis and pedicels (218.3 mg/kg f.w) were found in Malverina cultivar.

The content of rutin in whole berries as determined by Palomino *et al*. (2000) was 0.22 mg/kg f.w. while quercitrine was determined in traces only.

CONCLUSION

The contents of seven chemoprotective polyphenolic compounds were determined both in the grapes (berries) and in the rachis and pedicels (stems) of ten *Vitis vinifera* cultivars growing in southern Moravian vineyards, officially classified for the production of wine. For the determinations, the extraction with methanol and ethyl acetate and HPLC analysis of the extracts were used.

The contents of the individual polyphenolic compounds significantly differed in different various varieties. Among all the polyphenolic compounds studied, catechin and epicatechin were present in the highest contents both in berries and rachis and pedicels. The rachis and pedicels contained more catechin than the berries in all varieties studied. However, epicatechin was distributed more evenly in both berries and stems. Comparable levels of the chemoprotective polyphenolic compounds were observed in the white and blue vine grapes. Comparing our results with the corresponding literature values, we found similar contents of catechin, epicatechin, and gallic acid, and a different content of *trans*-resveratrol. Pterostilbene was found in the rachis and pedicels of three cultivars only, namely in Lemberger, St.Laurent, and Welschriesling (0.13, 0.11 and 0.10 mg/kg f.w., respectively) for the first time.

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Corresponding author:

Doc. Ing. Jan Tříska, CSc., Ústav systémové biologie a ekologie AV ČR, v.v.i., Branišovská 31,
370 05 České Budějovice, Česká republika
tel.: + 420 387 775 619, fax: + 420 385 310 347, e-mail: triska@usbe.cas.cz